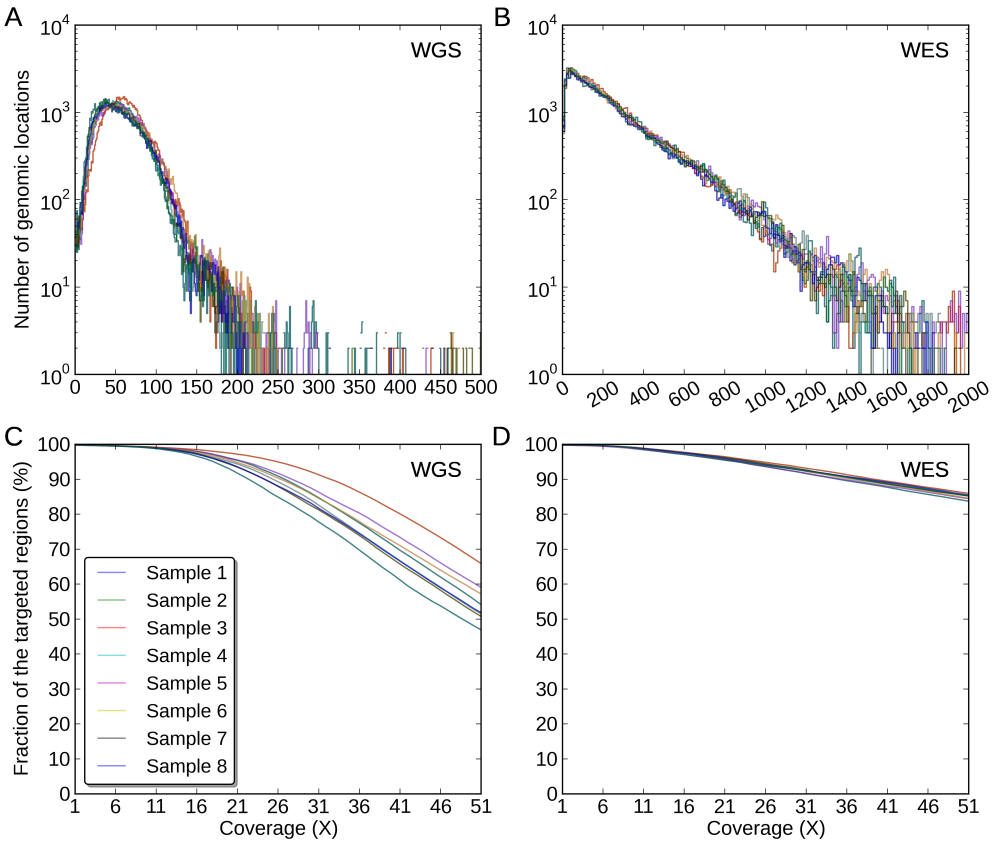
Additional Data File 1

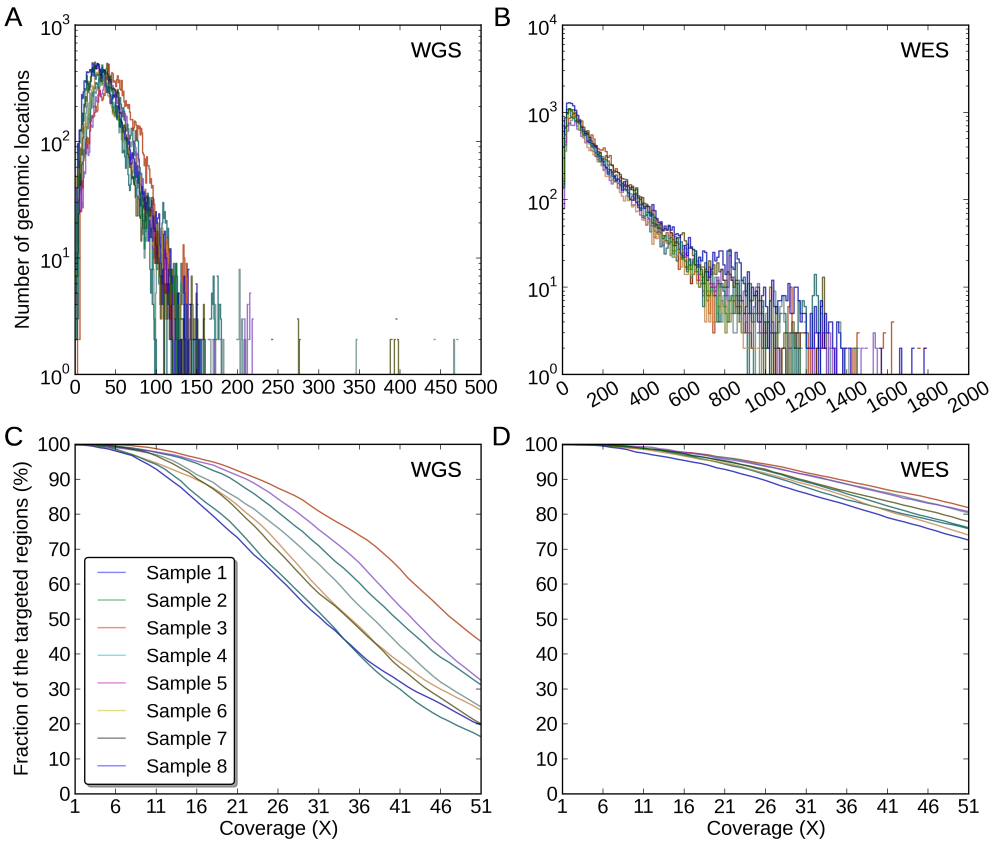
Supplemental Figures

Supplemental Figure S1



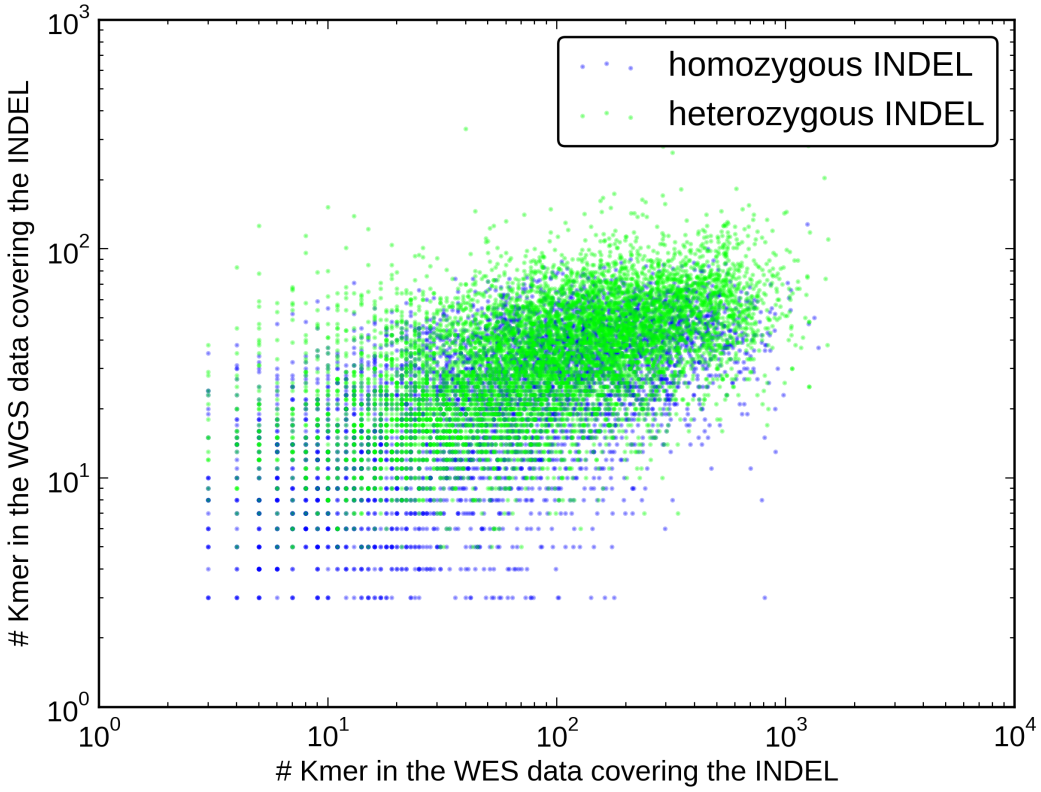
Supplemental Figure S1. **Coverage distributions of the WGS-WES intersection INDELs regions in (A) the WGS data, (B) the WES data**. The Y-axis for A) and B) is of log10-scale. The coverage fractions of the WGS-WES intersection INDELs regions from 1X to 51X in (C) the WGS data, (D) the WES data.

Supplemental Figure S2



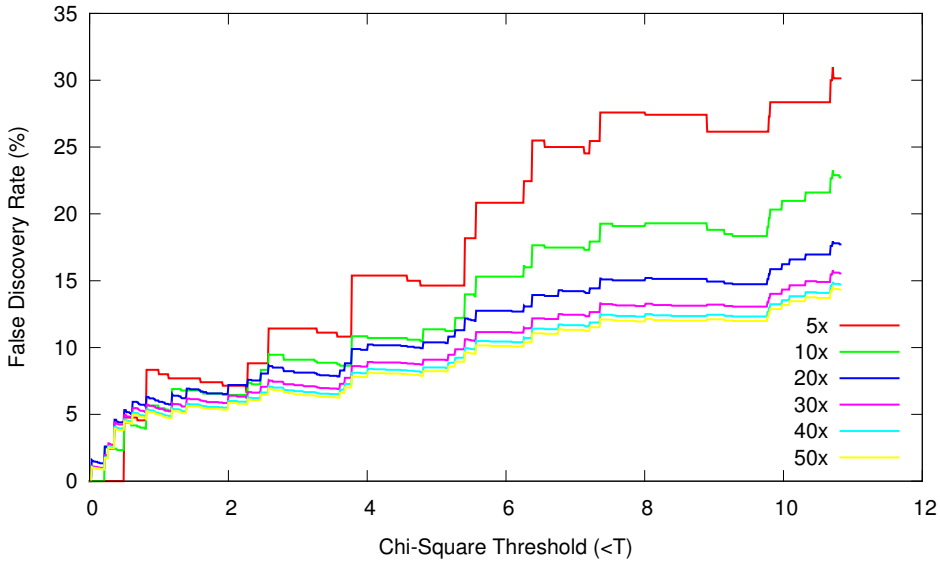
Supplemental Figure S2. **Coverage distributions of the WES-specific INDELs regions in (A) the WGS data, (B) the WES data**. The Y-axis for A) and B) is of log10-scale. The coverage fractions of the WES-specific INDELs regions from 1X to 51X in (C) the WGS data, (D) the WES data.

Supplemental Figure S3



Supplemental Figure S3. **Pair-wise base coverage relationship of INDEL called by both WGS and WES data**. These INDELs were partitioned by zygosities: homozygous (blue) and heterozygous INDELs (green). The X-axis shows the number of k-mer covering an INDEL in the WES data, and the Y-axis shows the number for WGS data.

Supplemental Figure S4



Supplemental Figure S4. **Characterization of the false discovery rate (FDR) based on validation data**. INDELs were partitioned based on k-mer coverage of the alternative allele and the INDEL Chi-Square scores. The X-axis shows Chi-Square scores of INDELs less than a certain threshold, and the Y-axis represents the FDR.

Supplemental Tables

Supplemental Table S1

Supplemental Table S1. **Mean depth coverage of WGS and WES data in different regions.** This table shows data corresponding to Figure 3, Figure 4, Supplemenatal Figure S1, and Supplemenatal Figure S2. The standard deviation is shown in parenthesis.

Mean coverage	Exonic targeted regions	WGS-WES intersection INDEL regions	WGS-specific INDEL regions	WES-specific INDEL regions
WGS	71X (3.3X)	58X (3.4X)	61X (2.9X)	41X (5.2X)
WES	337X (18.2X)	252X (7.0X)	137X (12.1X)	171X (10X)

Supplemental Table S2

Supplemental Table S2. **Mean coverage fractions of WGS and WES data in different regions.** This table shows data corresponding to Figure 3, Figure 4, Supplemenatal Figure S1, and Supplemenatal Figure S2. The standard deviation is shown in parenthesis.

	Coverage fraction	Exonic targeted regions	WGS-WES intersection INDEL regions	WGS-specific INDEL regions	WES-specific INDEL regions
WGS	1X	99.9% (0.1%)	99.8%(0.04%)	99.9%(0.03%)	99.9%(0.06%)
	20X	98.2% (0.2%)	96.0% (1.1%)	93.9% (1.4%)	86.9% (6.1%)
	50X	81.0% (3.1%)	57.5% (6.0%)	54.5% (0.4%)	29.4% (9.4%)
WES	1X	83.9% (1.1%)	99.8%(0.05%)	55.8% (0.3%)	99.9%(0.04%)
	20X	74.5% (0.1%)	96.6% (0.3%)	31.1% (2.1%)	96.0% (1.0%)
	50X	72.0% (0.3%)	85.7% (0.7%)	25.2% (3.7%)	78.7% (3.3%)

Supplemental Table S3

Supplemental Table S3. **Mean percentage and mean number of high quality, moderate quality, low quality INDELs in each call set.** This table shows data corresponding to Figure 5. The mean percentage and the mean number over eight

samples are shown in the upper and the lower of a cell, respectively. The standard deviation is shown in parenthesis.

	High quality	Moderate quality	Low quality
WGS-WES intersection INDELs	89% (0.7%) 1454 (11.7)	9% (0.5%) 148 (7.3)	2% (0.5%) 31 (8.3)
WGS-specific INDELs	78% (1.4%) 769 (13.9)	15% (1.1%) 151 (10.7)	7% (1.6%) 71 (15.8)
WES-specific INDELs	22% (3.4%) 71 (11.2)	37% (3.7%) 121 (11.9)	41% (3.3%) 133 (10.9)

Supplemental Table S4

Supplemental Table S4. **Mean percentages of high-quality INDELs partitioned by the following categories: homopolymer (A/C/G/T), other short tandem repeats (other STR), and non STR INDELs.** This table shows data corresponding to Figure 6. The standard deviation is shown in parenthesis.

Regions	WGS-WES intersection INDELs	WGS-specific INDELs	WES-specific INDELs
Poly-A	11.2% (0.8%)	13.6% (0.6%)	24% (3.0%)
Poly-C	0.09% (0.06%)	0.3% (0.1%)	1.4% (1.0%)
Poly-G	0.3% (0.09%)	0.5% (0.1%)	0.6% (0.8%)
Poly-T	9.0% (0.6%)	7.9% (0.7%)	30% (3.5%)
Other STR	9.6% (0.5%)	11.1% (0.9%)	12.5% (3.1%)
Non-STR	70% (1.2%)	67% (1.1%)	31.9% (6.1%)

Supplemental Table S5

Supplemental Table S5. **Mean fractions of low-quality INDELs partitioned by the following categories: homopolymer (A/C/G/T), other short tandem repeats (other STR), and non STR INDELs.** This table shows data corresponding to Figure 6. The standard deviation is shown in parenthesis.

Regions	WGS-WES intersection INDELs	WGS-specific INDELs	WES-specific INDELs
Poly-A	19.6% (12.5%)	26.1% (7.0%)	41.5% (3.2%)
Poly-C	0.6% (1.6%)	0% (0%)	0.3% (0.3%)
Poly-G	0%	0.4% (0.09%)	0.3% (0.3%)
Poly-T	24.6% (11.2%)	19.0% (5.6%)	41.1% (3.5%)
Other STR	21.0% (7.3%)	11.9% (3.8%)	6.2% (1.2%)

Non-STR	34.1% (14.1%)	42.6% (9.4%)	10.7% (2.5%)

Supplemental Table S6

Supplemental Table S6. **Number of INDELs in the WGS and WES data with multiple signatures partitioned by the following categories: homopolymer (A/C/G/T), other short tandem repeats (other STR), and non STR INDELs.** This table shows data corresponding to Figure 7. The standard deviation is shown in parenthesis.

Regions	WGS	WES
Poly-A	25 (5.7)	35 (6.0)
Poly-C	0.3 (0.4)	1 (0.3)
Poly-G	0.6 (0.5)	0.8 (0.7)
Poly-T	16 (4.0)	36 (5.3)
Other STR	25 (4.8)	24 (3.4)
Non-STR	9 (2.6)	6 (1.4)

Supplemental Table S7

Supplemental Table S7. **Number of reads in the following four regions: Exonic targeted regions, WGS-WES intersection INDEL regions, WGS-specific INDEL regions, WES-specific INDEL regions**.

Number of reads	Exonic targeted regions	WGS-WES intersection INDEL regions	WGS-specific INDEL regions	WES-specific INDEL regions
WGS	241984	49008	26417	1775
WES	815945	205698	44346	11251

Supplemental Table S8

Supplemental Table S8. **Probabilities of seeing k or more INDELs in a given family assuming a binomial distribution**. Here we assumed a binomial distribution of the de novo exonic INDELs in the 343 SSC families.

Number of INDELs	= 0	≥ 1	≥ 2
Probability	0.78	0.22	0.03

Number of INDELs	≥ 3	≥ 4	≥ 5
Probability	0.0020	0.0001	0.000005

Supplemental Table S9

Supplemental Table S9. **Putative *de novo* exonic INDELs in these two families before and after applying filtering critiria**. The number of INDELs within regions of homopolymer A (poly-A), homopolymer T (poly-T), and microsatellites (ms) are shown in the parenthesis.

Putative *de novo*	WGS (poly-A, poly-T, ms)	WES (poly-A, poly-T, ms)	WGS (After filtering)	WES (After filtering)
Family 1	45 (27,14,4)	5 (3,1,1)	0	0
Family 2	49 (24,22,3)	17 (8,5,4)	0	0

Supplemental Note 1

Analysis of the effect of new filtering criteria on *de novo* INDEL calls

The two families in this study were previously reported in a population-scale autism study, with Sanger validation of de novo calls [1]. We used the de novo mode of Scalpel to identify de novo INDELs in these two families again, resulting in one de novo call set for WGS data and another de novo call set for WES data per family. We partitioned each call set by regions and filtered out the low quality INDELs. Iossifov *et.al* 2012 reported a total of N=85 de novo exonic INDELs in 343 families, i.e. there was 0.1 de novo exonic INDEL per child [1]. If we assume a binomial distribution of the de novo exonic INDELs with an equal chance (p=1/343), the probability of seeing at least X de novo exonic INDELs in a given family in this study can be computed as below:

$$\mathrm{P}(\,\mathrm{X} \geq \mathrm{k}\ \mathrm{INDELs}) = \ 1 - \sum_{0}^{\mathrm{k}} \mathrm{P}(\mathrm{X} = \mathrm{k} - 1\,) = 1 - \sum_{0}^{\mathrm{k}-1} \binom{\mathrm{N}}{\mathrm{X}} \mathrm{p}^{\mathrm{X}} \mathrm{q}^{\mathrm{N}-\mathrm{X}}$$

where P($X \geq k$) is the probability of a given family having k or more de novo INDELs; N is the total number of exonic de novo INDELs reported, i.e. N=85; p is the probability of a hit on a given trial, i.e p=1/343; q=1-p.

Applications of using filtering criteria to reduce false positive de novo INDELs

Supplemental Table S8 shows the probabilities of seeing more than K INDELs from one of the 343 families reported in Iossifov *et al.* 2012 [1]. Scalpel has a de novo analysis mode; it could re-assemble each region associated with the candidate INDELs across the family members using a more sensitive parameter setting. This setting was indeed more sensitive for detecting de novo INDELs than single-sample calling. Due to this, we used the following more rigorous filtering criteria than the above assessment to exclude any

spurious false-positive de novo INDELs: coverage of the alternative allele >10 and Chi-Square score <4. Supplemental Table S9 showed the number of putative de novo INDELs in two families before and after applying this filtering criteria. All of the spurious *de novo* varaints in the two families were successfully excluded, which was consistent with the validated results in the variant database reported by Iossifov *et al.* 2012 [1]. We noticed that, in both families, the majority of these false-positive de novo INDELs were poly-A/T relevant (91% for WGS, 78% for WES), which was consistent with the above assessment. This suggested that if we used very sensitive callers, we should control for poly-A/T false-positive de novo INDELs by applying a more rigorous filtering criteria, especially in population-scale sequencing projects, where there is substantial expense with experimental validation.

References:

1. Iossifov I, Ronemus M, Levy D, Wang Z, Hakker I, Rosenbaum J, Yamrom B, Lee YH, Narzisi G, Leotta A, et al: **De novo gene disruptions in children on the autistic spectrum.** *Neuron* 2012, **74:**285-299.